Am. J. Hum. Genet. 61:785–789, 1997

GENE THERAPY '97
The Promise and Reality of Cancer Gene Therapy

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A variety of novel strategies for treating solid tumors by gene therapy have been developed in recent years. A significant limitation in many of these methods is the ability to transfer therapeutic genes uniformly into all tumor cells in situ. To increase cell killing within tumors and render metastatic disease managable, new strategies will be needed to deliver therapeutic genes systemically or to induce systemic antitumor responses following local therapy. Potential shortcomings of these approaches may be addressed through the positive phenomenon of a "bystander effect" to broaden the destructive effects of a given therapy. Bystander effects may act locally, via cell-cell contact, or systemically, through immune-mediated responses. This short review will explore the ability of the three most widely studied approaches to cancer gene therapy and will critically evaluate each one's ability to affect local growth and metastases and to provoke a defined bystander effect.

Tumor-Suppressor Gene Therapy

Cancer gene therapy in its purest form is the replacement, with a correct copy, of a gene whose mutation initiates or significantly alters the malignant phenotype. Cell death may not be the sole goal of such treatment: changes in growth, behavior, invasiveness, or metastatic ability represent other important goals in cancer containment. Since *p53* is the most commonly mutated gene in human cancer and influences transcription, cell-cycle arrest, DNA repair, and apoptosis (Harris 1996) as well as angiogenesis (Dameron et al. 1994), it has received the most interest as a target for cancer gene therapy. Transduction of cancer cells with *p53* can significantly inhibit growth and angiogenesis or can induce apoptosis in *p53* mutant cells in several tumor models, including lung and breast (Fujiwara et al. 1994; Lesoon-Wood et al. 1995; Xu et al. 1997). Enthusiasm has continued after a phase I clinical trial where injection of lung tumors with a *p53* retrovirus was nontoxic and suppressed tumor growth in six of nine patients (Roth et al. 1996).

However, the field of tumor-suppressor gene therapy is limited by the paucity of identified target genes known to induce or maintain the malignant phenotype. Furthermore, in preclinical in situ studies, eradication of treated tumors is a rarity, given the difficulty in transduction of sufficient numbers of cells within a cancer to facilitate a cure. A bystander effect, whereby more cells die than are transduced, has been proposed, but neither its significance nor a specific mechanism(s) has yet been identified (Fujiwara et al. 1994; Lesoon-Wood et al. 1995; Xu et al. 1997). Mutant or absent *p53* status has been associated with resistance to radiation therapy and to apoptosis-inducing chemotherapy (Lowe et al. 1994). Indeed, the combination of *p53* gene transduction with radiation or chemotherapy has resulted in local tumor control superior to either therapy alone (Gjerset et al. 1995; Ngyuyen et al. 1996) and is currently under investigation in clinical trial.

Direct suppression of metastatic growth through *p53* gene therapy has been attempted through systemic delivery of a liposome complex in a breast cancer model (Lesoon-Wood et al. 1995) or via hepatic-artery infusion for colonic liver metastases (Bookstein et al. 1996), although both approaches lack tumor targeting. A novel strategy to address the treatment of *p53* mutant metastases has been the development of an adenovirus that replicates only within *p53* mutant cells, killing through cell lysis (Bischoff et al. 1996). Although not truly gene therapy, systemic inoculation of this vector has resulted in significant growth suppression of a primary tumor (Heise et al. 1997) and deserves further investigation.

Suicide Gene Therapy

Suicide gene therapy is defined as the transduction of a gene that converts a pro-drug into a toxic substance; independently, the gene product and the pro-drug are nontoxic. Two such systems have been widely investigated: the *Echerichia coli* cytosine deaminase (*CD*) gene plus 5-fluorocytosine (5-FC) and the herpes simplex virus thymidine kinase gene (HSV-*tk*) plus ganciclovir (GCV).

Received July 23, 1997; accepted for publication August 19, 1997.

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This article represents the opinion of the authors and has not been peer reviewed.

0002-9297/97/6104-0002$02.00

The *CD* gene converts 5-FC to the chemotherapeutic agent, 5-fluorouracil (5-FU) (Huber et al. 1993) and has been studied centrally as a treatment for hepatic metastases of gastrointestinal tumors, for which 5-FU is commonly used. A significant bystander effect is active through production of locally high levels of freely diffusible 5-FU (Trinh et al. 1995). Systemic therapy with 5-FC results in growth suppression of *CD*-transduced tumors, whereas little suppression is achieved in the same tumors with high doses of systemic 5-FU (Huber et al. 1993). No systemic growth suppression was seen in nontransduced tumors growing in the same animals, indicating the lack of serum 5-FU levels sufficient for antitumor activity. However, other investigators have noted that successful treatment of *CD*-transduced tumors with 5-FC can result in activity against challenge tumors (Mullen et al. 1994). Depletion of $CD8^+$ T-cells or granulocytes abrogates the effects of *CD* plus 5-FC (Consalvo et al. 1995) in poorly immunogenic models, indicating possible immunological activity in this system.

Strategies for treating liver metastases have focused on regional delivery of the *CD* gene into areas surrounding metastases (Ohwada et al. 1996. Further refinements for systemic gene delivery are being explored through the use of tissue-specific promoters, such as carcinoembryonic antigen (*CEA*) or *a-fetoprotein* genes, for tumor targeting after hepatic-artery infusion of the *CD* vector (Richards et al. 1995; Kanai et al. 1997). However, outstanding issues with this approach include the development of resistance to 5-FU and the degree of killing in tumors resistant to 5-FU.

HSV-*tk* phosphorylates GCV, converting it to a nucleoside analogue that inhibits DNA synthesis (Moolten 1986). This metabolic change causes a significant bystander effect through several mechanisms: gap junctions transport nondiffusible phosphorylated GCV to nontransduced cells; nontransduced cells endocytose debris containing phosphorylated GCV from dying cells; and an induced immune response leads to tumor killing (Vile et al. 1994; Elshami et al. 1996*b;* Hamel et al. 1996; Mesnil et al. 1996). This therapy has been explored for a variety of cancers, including localized brain tumors (Culver et al. 1992; Barba et al. 1994; Chen et al. 1994*b*) and mesotheliomas (Elshami et al. 1996*a*), liver metastases (Caruso et al. 1993), and peritoneal-based metastases (Tong et al. 1996; Yee et al. 1996), leading to >35 clinical trials using this approach for human cancers worldwide. Although the growth-suppressive activities of HSV-*tk* plus GCV are significant, cure rates are low, undoubtedly because of in situ transduction inadequacies and the variability of the bystander effect. As with *p53* gene therapy, both the *CD* and HSV-*tk* systems sensitize cancer cells to radiation, suggesting possible combination therapies to control advanced tumors (Kim et al. 1995; Khil et al. 1996).

Use of HSV-*tk* plus GCV for the treatment of metastatic disease presents several problems. Treatment of tumors with HSV-*tk* suppresses growth of tumors derived from challenge injections of the parental cell line, indicating the induction of systemic antitumor activity in some models (Barba et al. 1994; Vile et al. 1994). Some evidence exists that this suppression is mediated by immune cells (Vile et al. 1994; Yamamoto et al. 1997), but the significance and generality of these observations are largely unknown. Furthermore, systemic delivery of HSV-*tk* to target metastatic lesions through intravenous (Vile et al. 1994) or peritoneal (Tong et al. 1996; Yee et al. 1996) routes may lead to significant liver injury (Yee et al. 1996; Brand et al. 1997; Qian et al. 1997); tissue-specific vectors may be required for safe systemic delivery of this gene.

Immunomodulatory Gene Therapy

Immunomodulatory gene therapy has been explored as a method to provoke cellular immune responses to metastatic lesions. A tumor vaccine, a suspension of irradiated tumor cells that are transduced with a cytokine gene, is injected into the skin of a patient to stimulate a systemic immune response against tumor-specific antigens. In numerous preclinical cancer models, vaccination with tumor cells expressing IL-2, GM-CSF, or IFN-g can generate cellular immunological activity against challenge tumors (Fearon et al. 1990; Gansbacher et al. 1990*a,* 1990*b*) and, in many cases, can cure or significantly control the growth of preestablished local or metastatic tumors (Dranoff et al. 1993; Porgador et al. 1993*a,* 1993*b;* Vieweg et al. 1994). However, several problems need to be addressed if this approach is to be practical. First, few candidate tumor-specific antigens to act as recognition targets have been identified. Second, in some studies, antitumor activity is only active against relatively low tumor burdens, predicting a potential difficulty in treating patients with large volume, established metastatic disease. Furthermore, although vaccination therapy of subcutaneous tumors generates superior results, vaccination therapy of orthotopically placed tumors has not been as successful (Vieweg et al. 1994), indicating potential problems in inducing potent antitumor immunological activities throughout the body after subcutaneous vaccination. Last, this strategy involves the costly and time-consuming process of tumor harvesting, ex vivo culturing, transfection and selection, and outgrowth of sufficient cells for injection.

A combination of cytokine and costimulatory molecule vaccination shows promise as a way to increase the efficiency of tumor vaccines (Salvadori et al. 1995; Gaken et al. 1997). The most widely investigated costimulatory molecule, B7, interacts with the CD28 receptor on T-cells to enhance T-cell activation (Linsley

and Ledbetter 1993). Transfection of B7 alone into tumor cells inhibits tumor growth, through immunological activity (Chen et al. 1992; Townsend and Allison 1993). This activity may be present only in cell lines that are inherently immunogenic (Chen et al. 1994*a*) and may not necessarily lead to systemic immunity (Wu et al. 1995; Chong et al. 1996), but covaccination of B7 with IL-2 (Salvadori et al. 1995; Gaken et al. 1997) markedly enhances this activity and may define future approaches to the improvement of vaccination strategies.

To avoid the lengthy process involved in generating tumor vaccines, in situ strategies to invoke immunological activities have been proposed. Direct injection, into a tumor, of adenoviral vectors containing the IL-2 gene can lead to complete regression (Addison et al. 1995; Cordier et al. 1995). The identification of infiltrating macrophages and T-cells within regressing lesions has been interpreted as evidence of a cellular-mediated antitumor response, which may account for the observed activity against challenge injections of the parent cell line. IL-12 has also attracted interest as an in situ agent, because it augments T-cell and natural killer–cell activities, induces IFN-g production, and promotes the differentiation of uncommitted T-cells to Th1 cells (Hendrzak and Brunda 1995). Vector-mediated delivery of IL-12 into established tumors suppresses tumor growth (Caruso et al. 1996) and can induce activity against challenge tumors (Bramson et al. 1996). However, both the cellular mechanisms responsible for these activities and the degree of activity induced against preestablished metastatic disease will need to be addressed.

The addition of IL-2 transgene therapy to HSV-*tk* plus GCV significantly inhibited growth of injected colon tumors, whereas vector delivery of either agent alone did not. This inhibitory activity appears to be mediated through the induction of tumor-specific $CD8^+$ T-lymphocytes (Chen et al. 1995). Whereas this immunity was relatively short lived, the addition of vector-mediated GM-CSF resulted in long term cellular-mediated, antitumor activity and the cure of 25% of treatment animals (Chen et al. 1996). In this model it is thought that (*a*) the HSV-*tk* activity induces rapid necrosis, which generates large quantities of tumor antigen, (*b*) GM-CSF allows this antigen to be presented more efficiently, and (*c*) IL-2 stimulates T-cell proliferation. Although still in its infancy, this strategy has the flexibility to develop combinations of cytokines necessary to induce antitumor activity both within the injected tumor and systemically.

Conclusion

The strategies presented in this review have made significant advances against local and metastatic tumor growth, but the future success of cancer gene therapy will hinge on the mastery of in situ transduction and of techniques to induce transgene expression or antitumor responses at a systemic level. While defined bystander effects enhance tumor killing, further evolutions in vector development, a thorough understanding of tumor and cellular immunology, and the expansion of therapies that restrict angiogenesis will be necessary for advancement of the course of cancer gene therapy.

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